Basic Enzymology (Part I)

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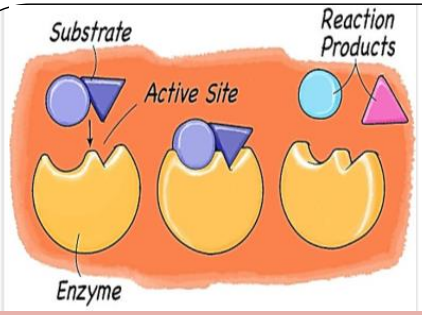

Biochemistry One

Lecture 6

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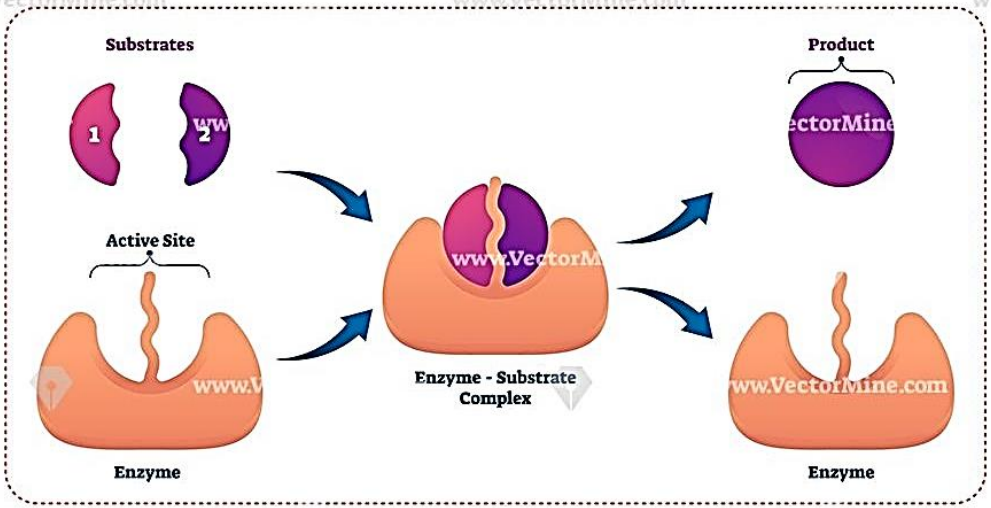
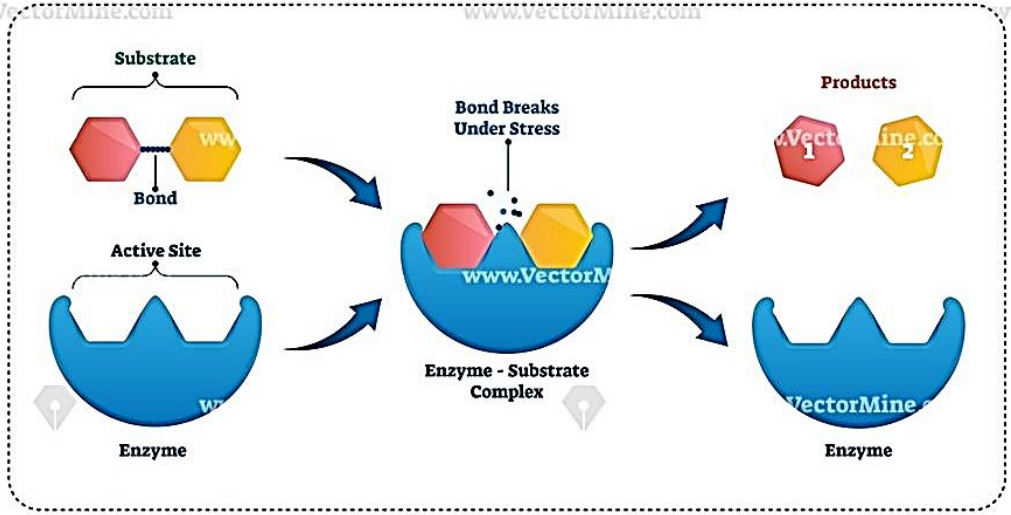
Basic Enzymology (Part I)





Objectives:

ENZYME

By the end of this lecture you should be familiar with:

- Definition, properties and nomenclature of enzymes.
- Classification of enzymes.
- Mechanism of enzyme action.
- Factors affecting enzyme activity.
- Enzyme kinetics.
- Types of enzyme activation.
- Mechanism of enzyme activation.

Definition & Properties of the Enzymes:

- En = inZyme = yeast.
- **Enzymes**: they are **protein** biocatalyst that accelerate the rate of chemical reactions (103–108 times faster) at **low** or normal temperature and low concentrations of reactants.

They have the following common features:

1. All are produced by living cells and can act outside these cells.
2. They are needed in very small amounts.
3. Proteins of high molecular weight and affected by heat.
4. Catalyze only one type of chemical reaction.
5. They accelerate the reaction without affecting its equilibrium (decrease the energy needed for activation).
6. They are not changed chemically by the end of the reaction.
7. Highly specific (e.g., act on a specific substrate).
8. Most of enzymes are intracellular therefore, measuring of some enzymes in plasma is useful for diagnosis of diseases.

Enzyme Nomenclature:

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1-Trivial name: such as trypsin, pepsin, and chymotrypsin

2-Recommended name: short and convenient for everyday use by adding the suffix **ase** to the name of substrate;

- Substrate + *-ase* (hexokinase, glucokinase, Maltase, lactase and sucrase).
- Action performed + *-ase* (dehydrogenase, oxygenase).

3-Systematic name: the reaction specificity + the substrate specificity.

- Each enzyme is entered in the *Enzyme Catalogue* with a four-digit Enzyme Commission number *(EC number)*.
- The first digit → class number (6 major classes).
- The second digit → functional group upon which the enzyme acts (subclass).
- The third digit → coenzyme (sub-subclass).
- The fourth digit → substrate (order of enzyme in sub-subclass).

Classification of Enzymes:

1-Oxido-reductases:

- catalyzes oxidation-reduction reaction between two substrates. The mechanism of oxidation is either **addition of oxygen (oxidase)** or **removal of hydrogen (dehydrogenase)**.
- **Oxidase** catalyzes transfer of electron or hydrogen from substrate to oxygen e.g.:-glucose oxidase that convert glucose to gluconate and H_2O_2
- **Oxygenase**: catalyze incorporation of oxygen molecule into substrate.
- **Mono-oxygenase**: catalyze the incorporation of one oxygen atom into substrate.

2-Transferases:

- Catalyzes the transfer of a group other than hydrogen (as acyl, amino, and phosphate) between two substrates.

Classification of Enzymes:

ENZYME

3-Hydrolases:

- Catalyzes hydrolysis of substrate (i.e. breakdown of the chemical bond by addition of water) such as digestive enzyme, and peptidases.

4-Lyases:

- Catalyzes removal of group from substrate by mechanism other than hydrolysis e.g.: aldolase enzyme which convert fructose-1,6-diphosphate into glyceraldehydes-3-phosphate and DHAP .

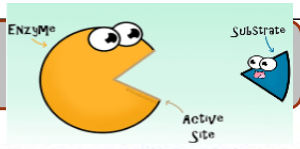
5-Isomerases:

- Catalyzes the **interconversion** of one isomer to the other e.g. glucose 6-p is converted into fructose -6-p by isomerase.

6-Ligases:

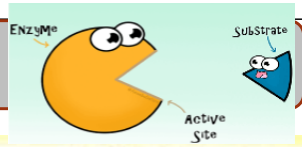
- Catalyzes the joining of 2 substrates using high energy released by hydrolysis of high energy bond of ATP e.g.: Pyruvic acid is joined to CO_2 forming oxaloacetic acid by aid of carboxylase enzyme.

Classification of Enzymes:



Main group	Subgroup	Action
1- Oxido -reductases	Dehydrogenases	- transfer hydrogen from the substrate to another hydrogen carrier
	Oxidases	- transfer of hydrogen from the substrate using oxygen as a hydrogen acceptor
	Reductase	- add hydrogen to the substrate using NADPH as a hydrogen donor
	Hydroperoxidases	- transfer hydrogen from substrate to hydrogen peroxide forming water
	Oxygenases	- add one or two atoms of oxygen
2- Transferases	Kinases	- transfer of a phosphate group from ATP ,or a relative compound, to another compound
	Aminotransferases	- transfer of amino group from α-amino acid to α-keto acid forming a new α-keto acid and a new α-amino acid
	Transmethylases	- transfer of a methyl group from a methyl donor to a methyl acceptor

Classification of Enzymes:



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3- Hydrolases	Glycosidases	- add water across a glycosidic bonds to cleave it
	Esterases	- add water across an ester bond to cleave it
	Peptidases	- add water across a peptide bond to cleave it
4- Lyases	Decarboxylases	- removal of carbon dioxide
	Dehydratases	- removal of water
	Aldolases	- splitting of an aldehyde from an alcohol group
5- Isomerases	Mutases	- transfer of a group from one atom to another in the same molecule
	Epimerases	- interconversion of two epimers
6- Ligases	Synthases	- Join molecules together without the use of ATP
	Synthetases	- Join molecules using ATP as a source of energy

Classification of Enzymes:

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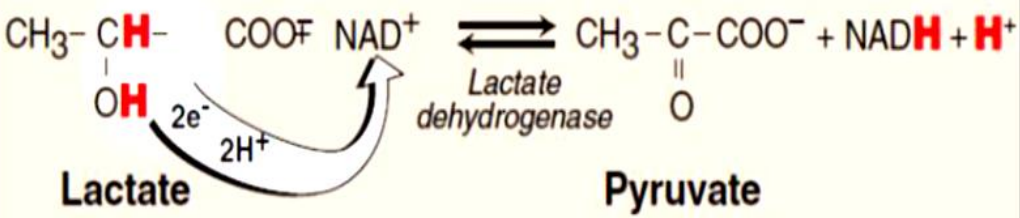
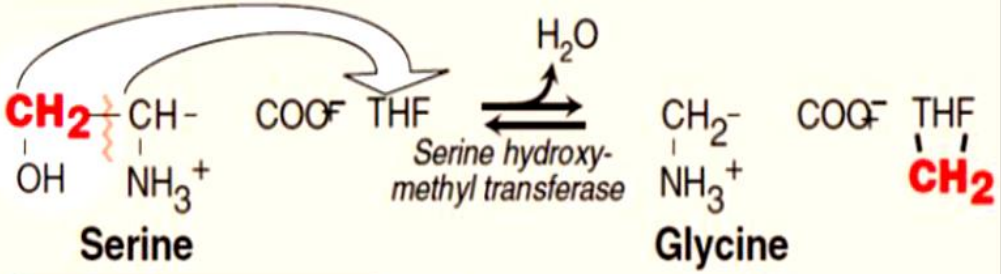
1. *Oxidoreductases*

Catalyze oxidation-reduction reactions, such as:



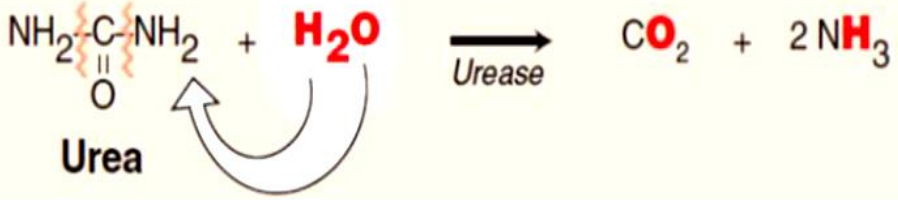
2. *Transferases*

Catalyze transfer of C-, N-, or P-containing groups, such as:



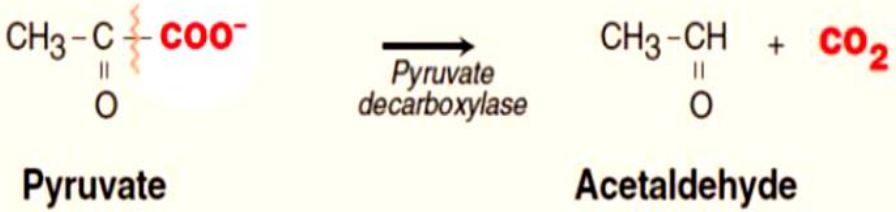
3. *Hydrolases*

Catalyze cleavage of bonds by addition of water, such as:



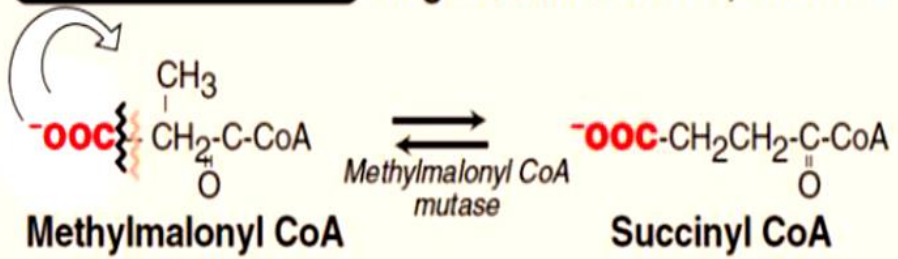
4. *Lyases*

Catalyze cleavage of C–C, C–S and certain C–N bonds, such as:



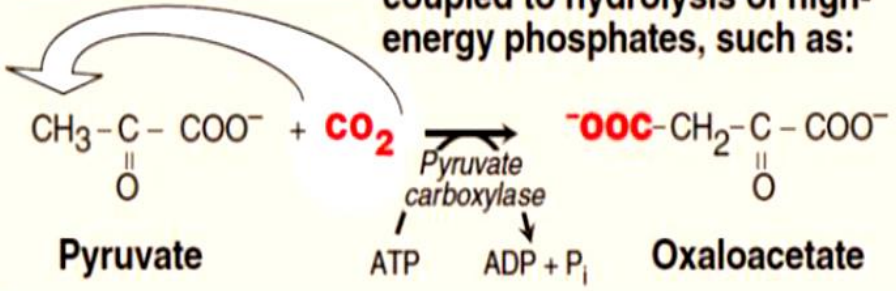
5. *Isomerases*

Catalyze racemization of optical or geometric isomers, such as:



6. *Ligases*

Catalyze formation of bonds between carbon and O, S, N coupled to hydrolysis of high-energy phosphates, such as:



Mechanism of Enzyme Action:

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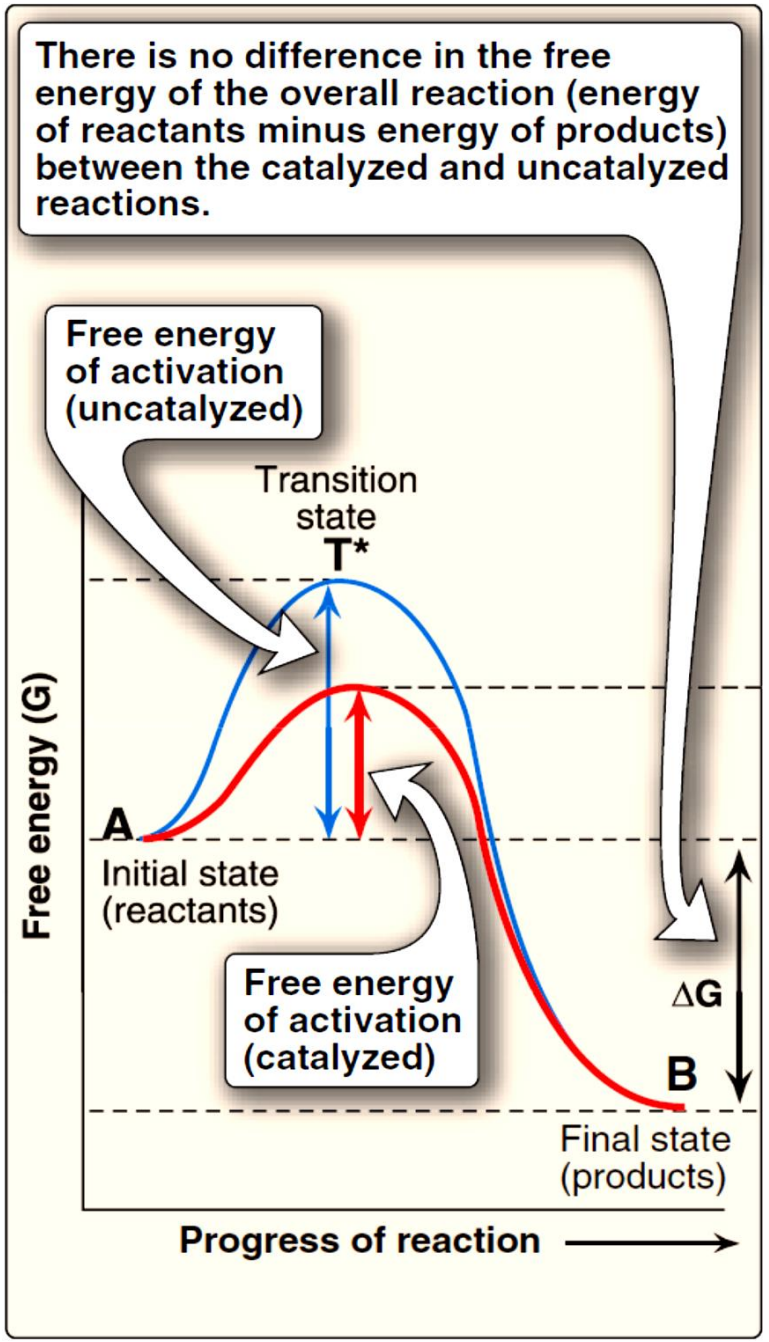
- Enzymes are large protein molecules. Each contains a small specific region, which interacts with the substrate, termed the active site, substrate site or catalytic site.
- Enzymes increase the rate of chemical reactions by decreasing the energy needed for substrate activation.
- Generally certain amino acid side chains have important role in enzyme catalysis e.g. SH of cysteine, OH of hydroxy-amino acids, carboxylic group of acidic amino acids and amino group of basic amino acids.
- They help in binding of the enzyme with its substrate and with the groups undergoing transfer (added or removed).



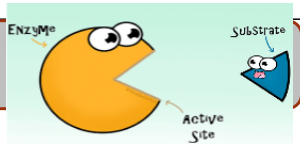
Mechanism of Enzyme Action:

ENZYME

The Energy changes during the reaction:

- All chemical reactions have an energy barrier separating the reactants and the products.
- **This barrier**, called the free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product.
- For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state.
- Enzyme accelerates the rate of reaction by *lowering the free energy of activation*.
- **The theories of enzyme action can be explained by the following two models:**

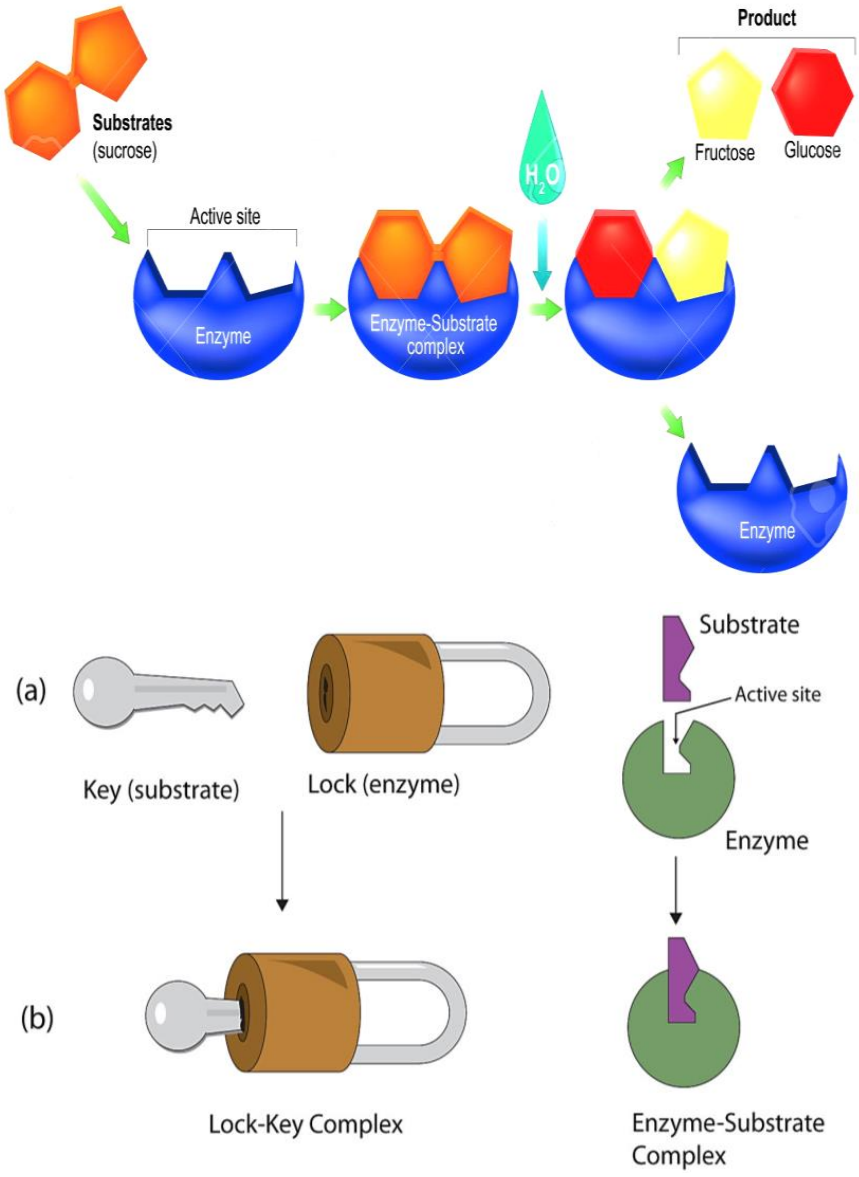
Mechanism of Enzyme Action:



"LOCK and KEY" model
(degradation)

1-The key and lock (Fisher model) theory:

- The active site of the enzyme is complementary in conformation to the substrate, so that enzyme and substrate recognize each other. This theory postulates that active site has fixed shape.
- The substrate binds to a specific site on the enzyme to from enzyme substrate complex, this is followed by activation of the substrate, then formation of the reaction products and the enzyme sets free to catalyze a new reaction.
- The only disadvantage of this model is the rigidity of active site.



Mechanism of Enzyme Action:

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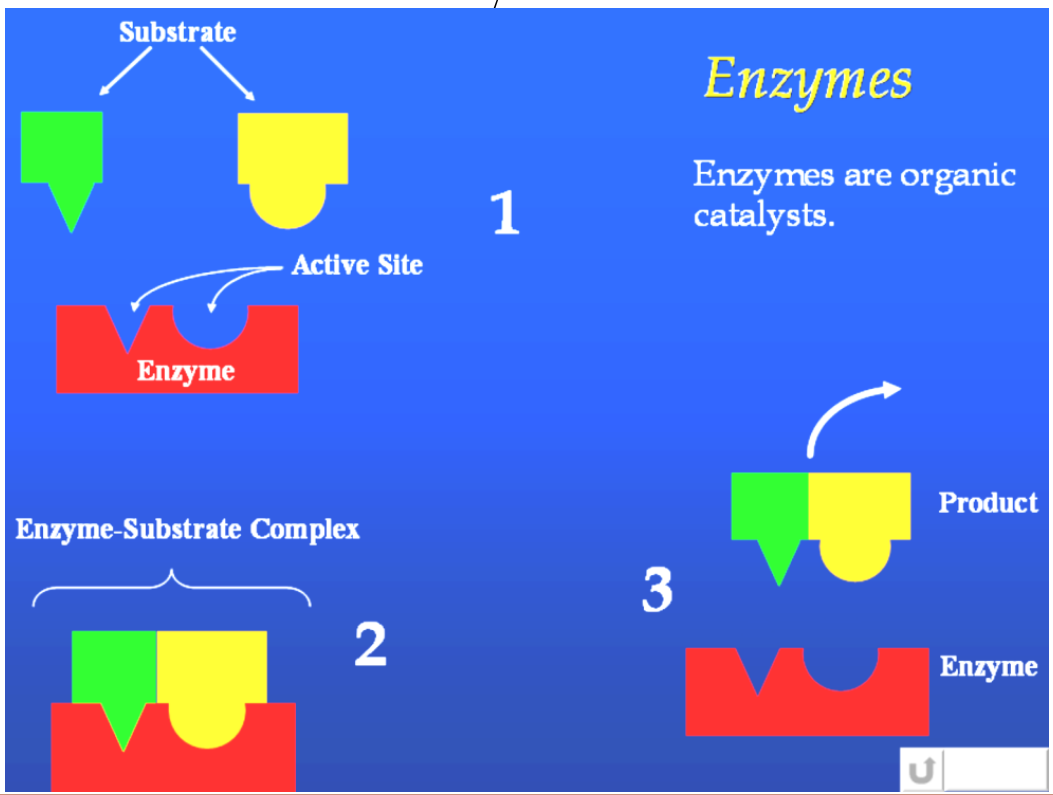
2-The induced fit theory (Khoshland model):

- The enzyme changes its shape upon binding the substrate, so that catalytic site is suggested to be pre-shaped to fit with substrate.
- In this model, the substrate induces a conformational changes in the enzyme, which make the catalytic site (or groups) more fit or more suitable for both binding of substrate and catalysis.



Factors Affecting Enzyme Activity:

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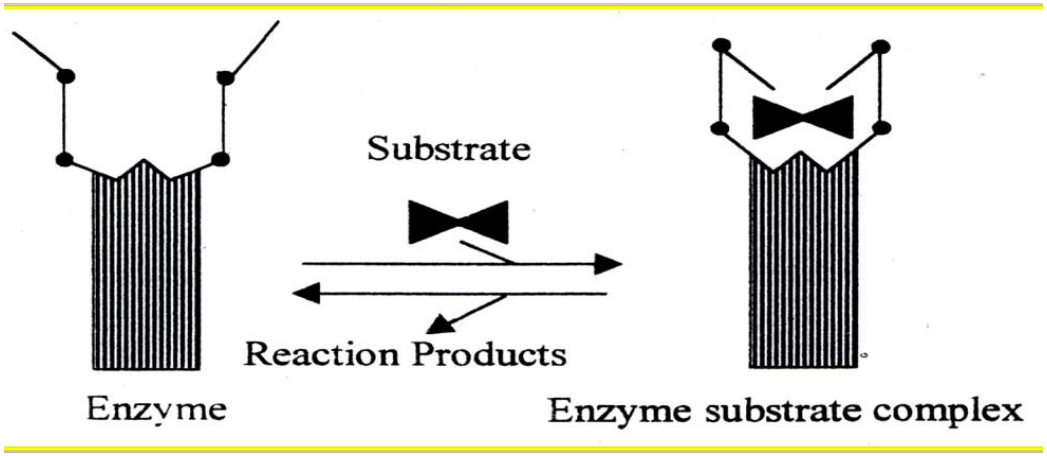
- The enzyme activity is measured by the rate of the reaction.
- **Rate of reaction (Turnover or velocity):** is the number of moles of substrate converted to product per second (mol/s).
- **1 Katal:** amount of enzyme required to increase the turnover by 1 mol/s.
- **IU:** amount of enzymes that catalyze conversion of 1 π mol of substrate to product per minute.



Factors Affecting Enzyme Activity:

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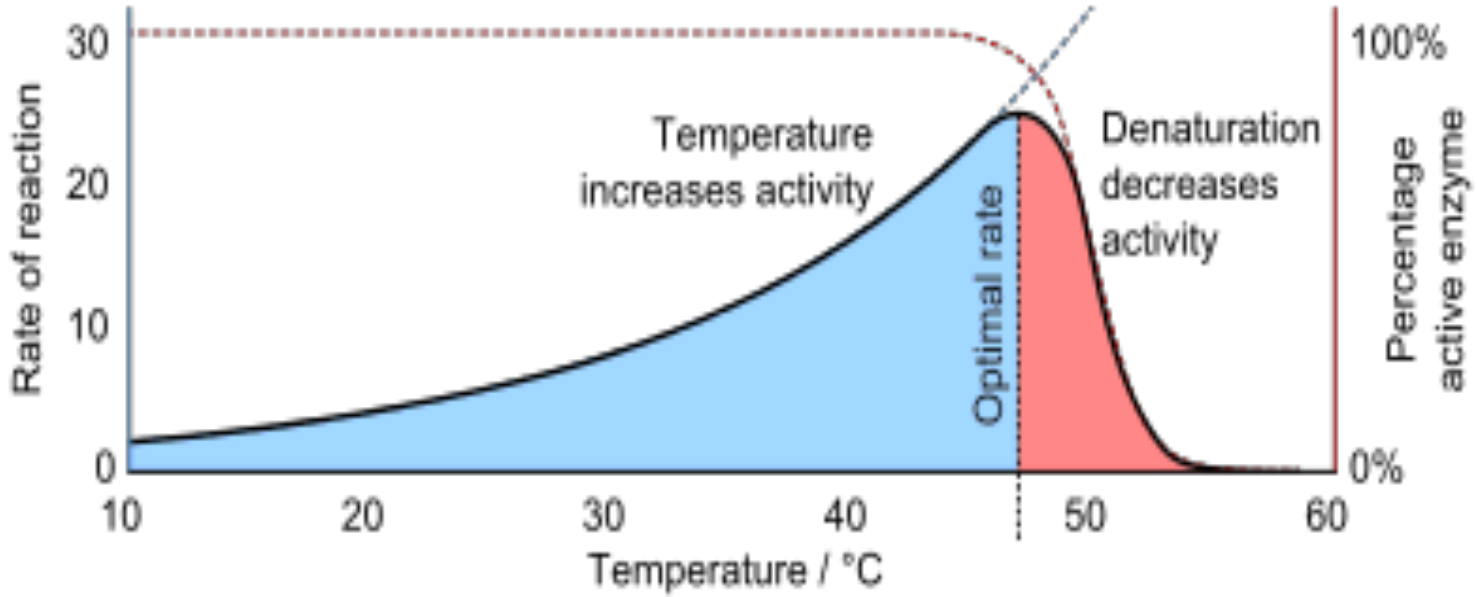
1-Temperature:

- The rate of an enzyme catalyzed reaction increases by raising temperature till it reaches a maximum activity at the optimum temperature (around 40°), after that the activity of the enzyme decreases.
- At first, the rise of temperature increases the kinetic energy of the molecules, then above optimum temperature, gradual **denaturation** of the enzyme occurs with complete loss of catalytic activity at 70°C.



Factors Affecting Enzyme Activity:

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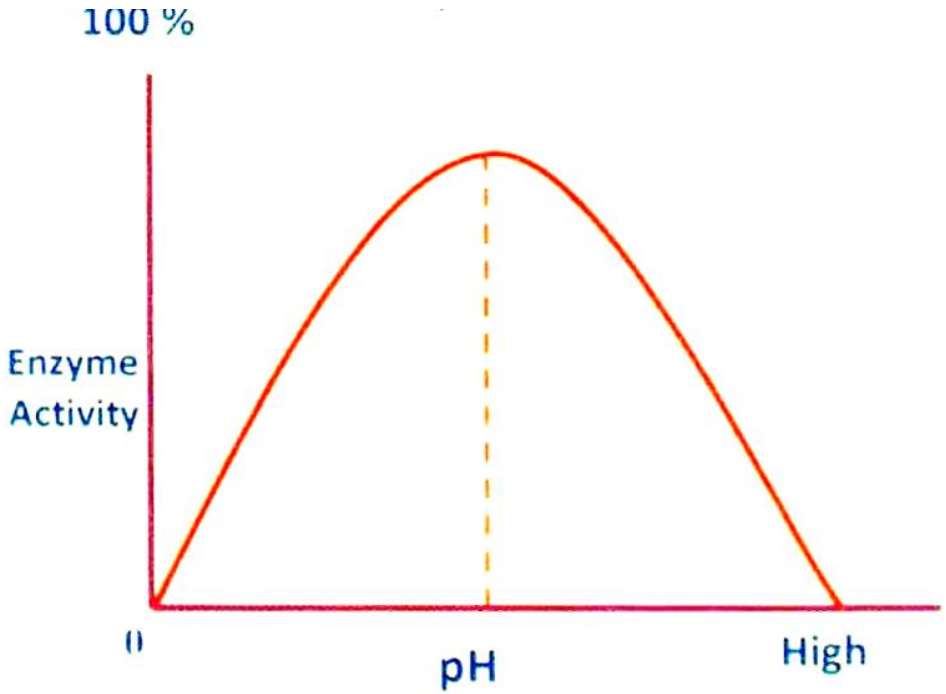
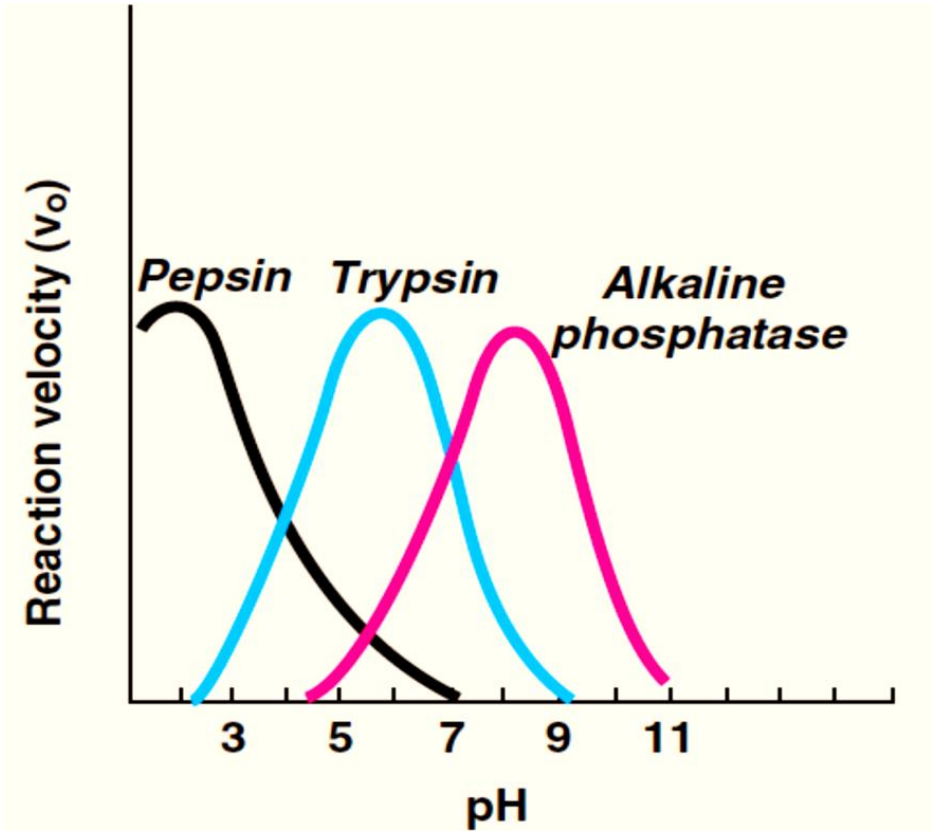
2-pH:

- Any enzyme has an optimum pH, at which it acts maximally. This pH usually ranges between 5 to 9. Marked changes in pH produces conformational changes in protein structures that result in decreased activity.



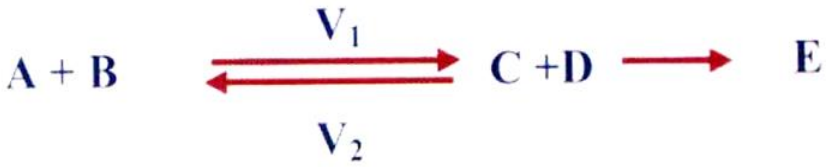


Factors Affecting Enzyme Activity:

ENZYME

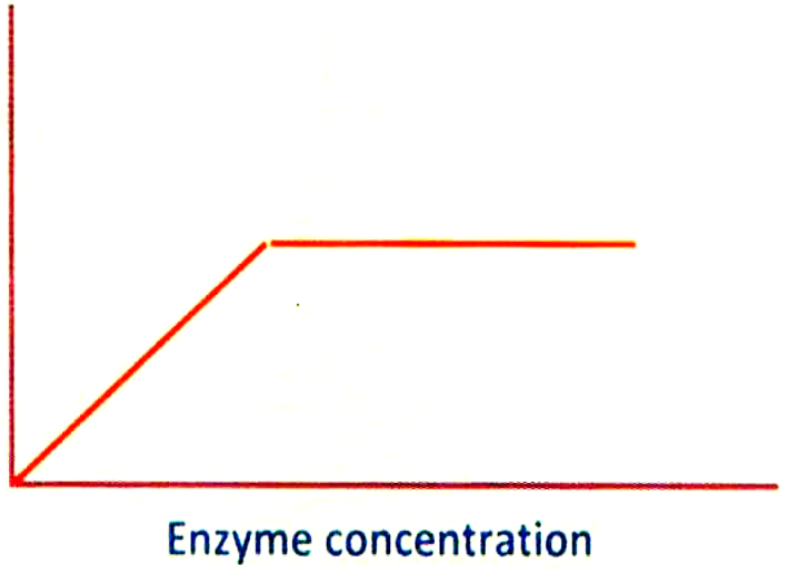
3- Reaction Products:

- For a reversible reaction, accumulation of the reaction products stimulates the reversal of the reaction (increases V_2) and produces a net decrease in V_1 till equilibrium is reached ($V_1 = V_2$).
- Also removal of reaction products (conversion of C + D into E) accelerates the initial reaction and increases V1 to maximum.



4- Enzyme Concentration:

- The velocity of the reaction increases as the enzyme concentration increases up to a certain point.
- Above this point the concentration of the substrate is a limiting factor.

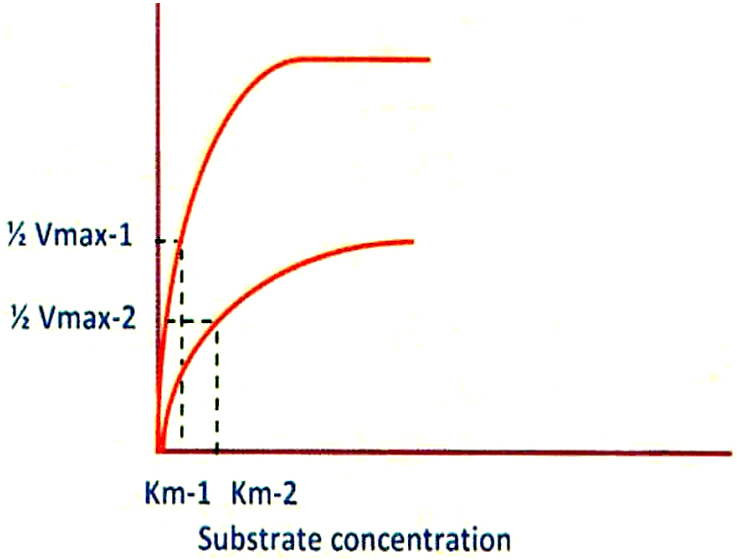


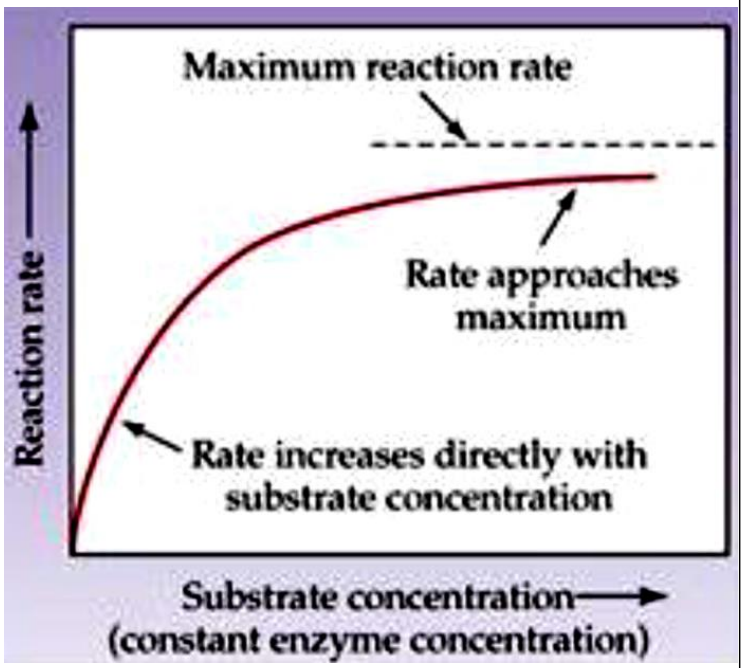
Factors Affecting Enzyme Activity:

ENZYME

5- Substrate Concentration:

- The velocity of the reaction increases as the substrate concentration increases up to a point where the enzyme is saturated with the substrate.
- The substrate concentration that produces half maximal velocity termed **Michaelis constant** (or Km).
- Enzymes with high affinity to substrate have low Km and vice versa.
- E.g. enzyme-1 has lower Km-1 and higher affinity to its substrate (high activity at low substrate concentration) compared to enzyme-2, which has higher Km-2 and lower affinity to its substrate (low activity at high substrate concentration).





Enzyme Kinetics:

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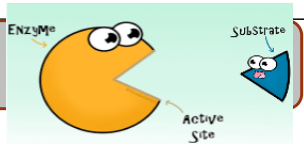
- It is the study of the rate or velocity of reactions catalyzed by enzymes.
- **Initial reaction velocity (V0):** The rate at which reaction proceeds and it is measured as decrease in concentration of substrates or increase in concentration of products with time.
- If the enzyme is incubated with its substrate and the appearance of the product is recorded as graph, the resulting line will have hyperbolic.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

where S is the substrate
E is the enzyme
ES is the enzyme–substrate complex
P is the product
k_1, k_{-1}, and k_2 are rate constants

Enzyme Kinetics:



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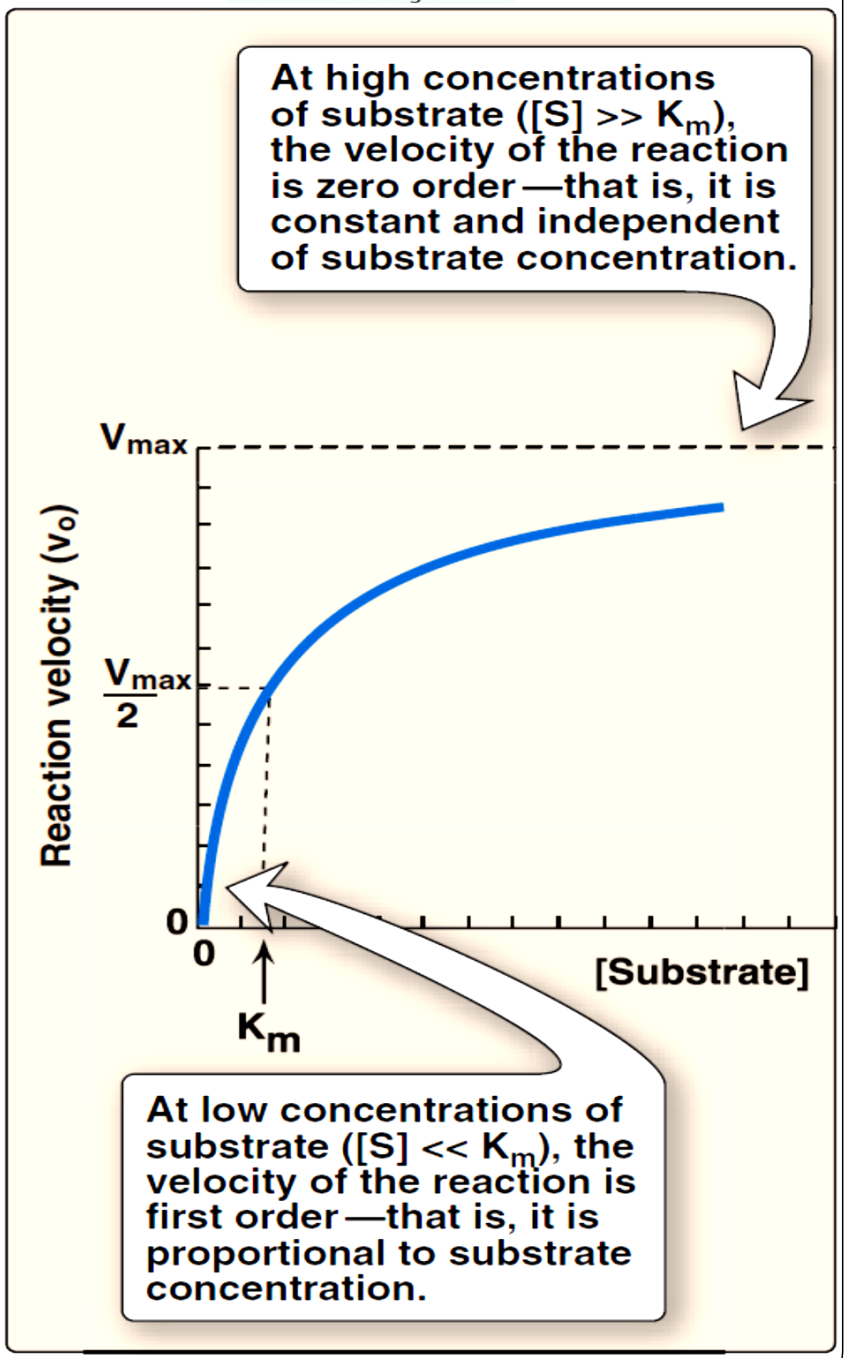
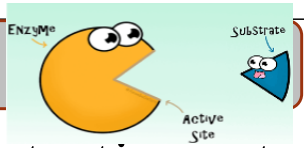
- If Km= [S] → the velocity will be ½ Vmax.
- Thus, **Km** can be defined as substrate concentration that produces half maximum velocity.

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

where
v_o = initial reaction velocity
V_{max} = maximal velocity
K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$
[S] = substrate concentration



Michaelis-Menten Equation:



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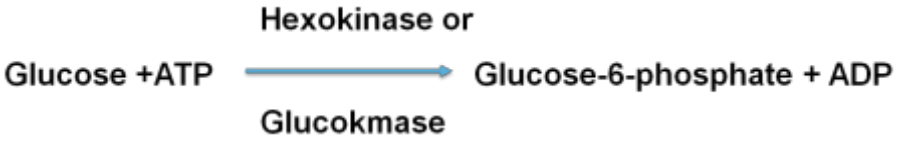
- **Km** is numerically equal to the substrate concentration at which the reaction velocity is equal to ½ Vmax.



- Substrates are usually present in physiological fluids in amounts nearly equal to K_m values.
- K_m is constant, characteristic for each enzyme and particular substrate.
- K_m reflects the affinity of the enzyme to substrate.
- Small (low) K_m = high affinity of the enzyme for substrate i.e. low concentration of substrate is needed to half saturate enzyme.
- Large (high) K_m = low affinity of the enzyme for the substrate (high concentration of substrate is needed to half saturate enzyme).
- Km does not vary with the concentration of enzyme.
- Examples; Hexokinase is more active than glucokinase because the amount of glucose (substrate) needed to produce half V_{max} in case of hexokinase is less (2 mg) than in case of glucokinase (200 mg) i.e. K_m of hexokinase is less than of glucokinase.

Determination of Km and Vmax for a given enzyme:

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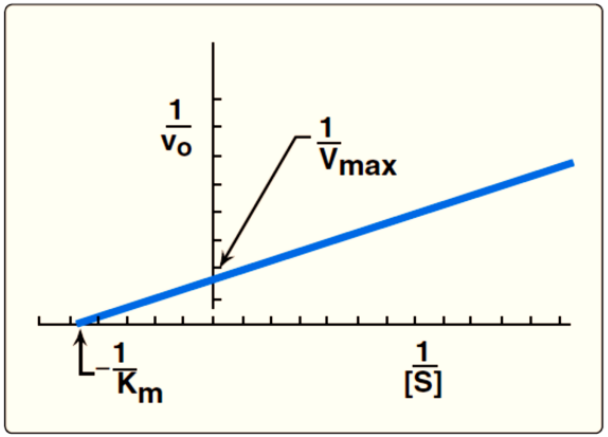
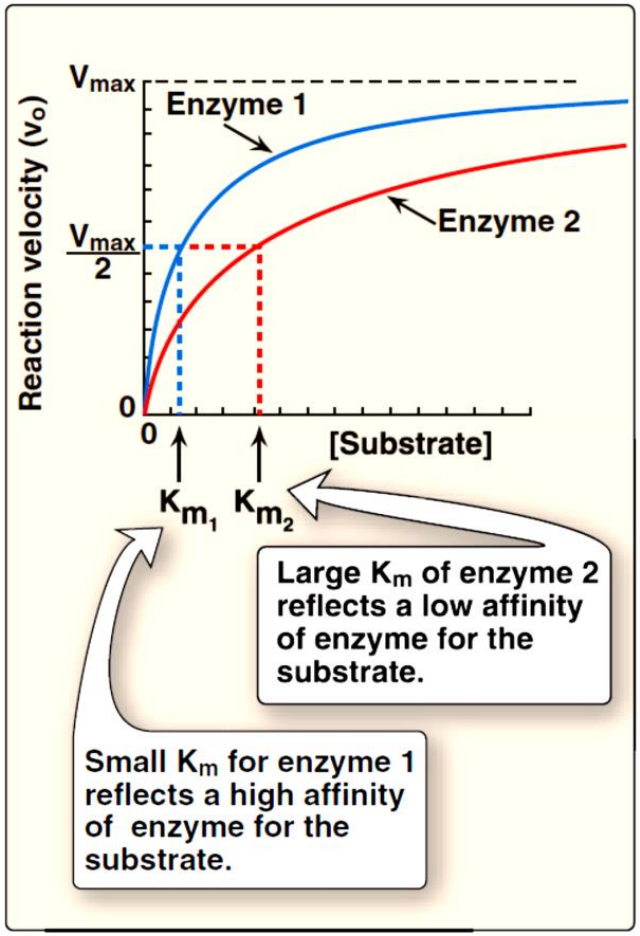
- Calculation of K_m and V_{max} to determine the mechanism of action of enzyme inhibitors.
- The Km value is usually expressed in units of concentration (moles/L).
- 1/V0 is plotted versus 1/[S], a straight line is obtained (Line weaver-Burk plot) and it becomes more practical to estimate both Km and Vmax.

The equation describing the Lineweaver-Burk plot is:

$$\frac{1}{v_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

where the intercept on the x-axis is equal to $-1/K_m$, and the intercept on the y-axis is equal to $1/V_{max}$.





Types of Enzyme Activities:

- They are mainly proteins in nature, either in the form of simple protein enzymes (Apoenzyme) or conjugated protein enzymes (Holoenzymes).
- **Holoenzymes** are formed of a protein part (apoenzyme/inactive alone) and a non-protein part (coenzyme or prosthetic group).
- **Coenzymes** are non-covalently (loosely) bounded to the apoenzyme and are low molecular weight organic molecules e.g. NAD^+ for lactate dehydrogenases. They act as carriers for certain groups, which have to be added to or removed from the substrate.
- **Prosthetic groups** are covalently (firmly) bounded to the apoenzyme. They are either organic e.g. heme in cytochromes, or inorganic e.g. metal ions as zinc in carbonic anhydrase.
- **Cofactor**: Metal ion Fe and Zn.

Coenzymes:

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Carriers are classified into **two** main groups:

1- Hydrogen and Electron Carriers: They include the following:

- NAD^+ and $NADP^+$.
- FMN and FAD.
- Heme in cytochromes.
- Lipoate.
- L-ascorbic acid.
- Glutathione.
- CoQ.

II- Other Group Carriers: They are vitamin derivatives and include the following:

- PLP (Pyridoxal phosphate) (NH_2).
- Biotin & TPP (CO_2).
- CoA (CoA-SH) (Acyl group).
- Tetrahydrofolate (CH_3).
- Folic acid = one carbon carrier. cobolamine= CH_3 carrier.

Mechanisms of Enzyme Activations:

Include the following:

1- Activation of zymogens.

2- Activation by metal ions.

3- Allosteric activation.

4- Covalent modification.

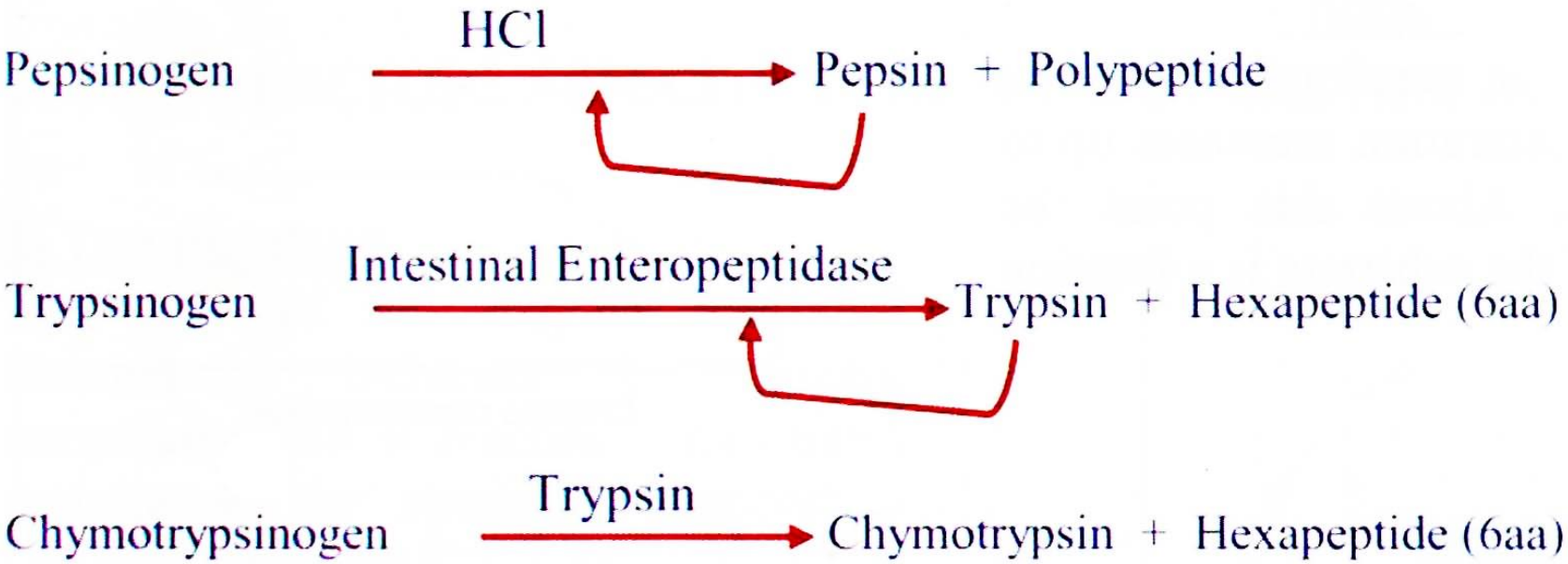
1- Activation of Zymogens (Pro-enzymes):

- Many enzymes are formed in the form of proenzymes or zymogens.
- In this form they are inactive. Activation requires proteolysis (removal of a part of the polypeptide chain which masks the active site or substrate site).
- A good example is the formation of digestive proteolytic enzymes as zymogens inside the cells to prevent digestion of cellular proteins.
- When these zymogens are released to the gut, they are activated to digest food proteins.
- Many of these enzymes after activation can activate its zymogen in a process termed autocatalytic activation (**autocatalysis**).

Mechanisms of Enzyme Activations:

ENZYME

- Another good example is the activation of different blood clotting factors.
- Many of these factors are formed as zymogens and activated by specific proteases.



Mechanisms of Enzyme Activations:

2- Metal Ion Activation:

- There are three main possible mechanisms by which the metals interact with the enzyme and substrate as follows:

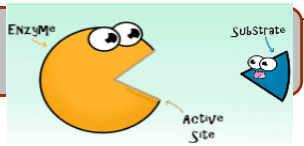
A. The metal helps to maintain an active conformation of the enzyme e.g. Mg^{2+} in glutamine synthase.

B. The metal binds with the substrate, then it helps binding of the substrate to the enzyme as in phosphotransferase reactions.

C. The metal is associated with the active center of the enzyme and helps in binding of the substrate to the enzyme e.g. cytochromes.

$$ATP + Mg^{2+} \xrightarrow{Enz} \text{ATP-Mg complex} \longrightarrow \text{Enz-ATP-Mg complex}$$

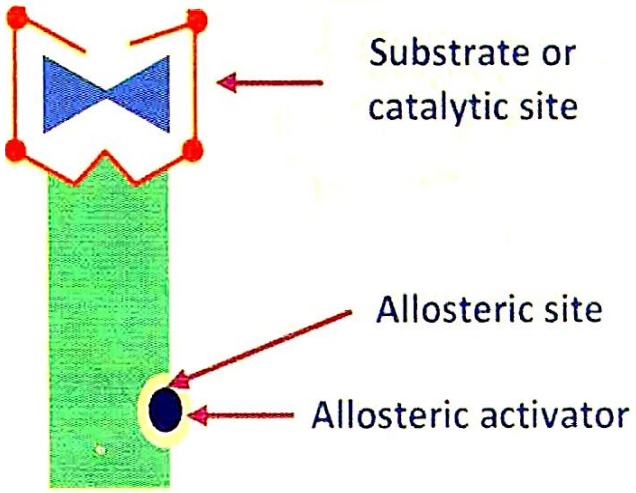
Mechanisms of Enzyme Activations:



ENZYME

3- Allosteric Activation (Non-Covalent Modification):

- Certain enzymes contain specific site (away from the catalytic site). The binding of an allosteric activator with the allosteric site produces conformational changes in the protein structure of the enzyme which result in increased velocity of the reaction.



4. Covalent modification: Phosphorylation and dephosphorylation(by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme).

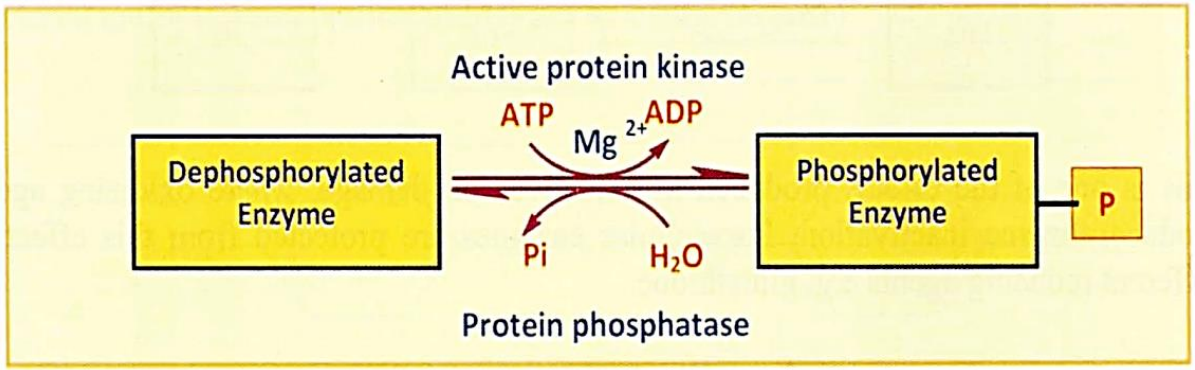
5. Induction and repression of enzyme synthesis: Cells can regulate the amount of enzyme present by altering the rate of enzyme degradation (repression) or, the rate of enzyme synthesis (induction).

Mechanisms of Enzyme Activations:

ENZYME

4- Covalent Modification for Activation by Phosphorylation and Dephosphorylation):

- Many enzymes are activated by phosphorylation and inactivated by dephosphorylation and vice versa.
- This means that the enzyme is present in two interconvertible forms (phosphorylated and dephosphorylated).
- The phosphate groups are usually attached to the hydroxyl group of amino acid residues (mainly serine or tyrosine) present in the polypeptide chain of the enzyme.
- Good example is the activation of glycogen phosphorylase and hormone sensitive lipase by phosphorylation and activation of glycogen synthase by dephosphorylation.



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